

Date: October 19, 2017
Protocol Title: Nicotine Dependence, Withdrawal and Replacement Therapy Assessed by PET Imaging
Principal Investigator: Mary E. McCaul, PhD
NCT: 01664741
Application Number: 00-02-03-05 / NA_00036996

Johns Hopkins Medicine - eForm A

1. Abstract

This protocol is an extension of previously completed studies examining the mu- and delta-opioid system in alcohol dependent and healthy control subjects. The current procedures extend this work by examining nicotine effects on the mu-opioid system during active smoking and nicotine replacement therapy. This work was previously approved in V5.0 – V5.12 of RPN 00-02-03-05. Funding for this study is pending from the NIH National Institute on Drug Abuse.

2. Objectives (include all primary and secondary objectives)

This protocol will:

1. Characterize the effects of cigarette smoking and nicotine replacement treatment (NRT) on the mu-opioid system

3. Background (briefly describe pre-clinical and clinical data, current experience with procedures, drug or device, and any other relevant information to justify the research)

The endogenous opioid system is involved in smoking initiation, nicotine craving and reward as well as nicotine withdrawal symptoms. Interestingly, research suggests that sexual dimorphic features of the endogenous mu-opioid system may in part explain gender differences in nicotine effects. To better understand the role of the mu-opioid system in poorer NRT responses in women, this proposal will examine nicotine replacement therapy (NRT) effects on mu opioid receptor binding potential (MOR BP) in female compared to male smokers during active (ANRT) versus placebo (PNRT) treatment.

Hypotheses:

1. We predict that MOR BP will be reduced under active smoking conditions compared to nonsmokers.
Hypotheses: 1a) We predict that female smokers will have lower MOR BP compared to male smokers during active smoking status. 1b) We predict that females smokers in active smoking status will have lower MOR BP compared with nonsmoking women. 1c) We predict that males smokers in active smoking status will have lower MOR BP compared with nonsmoking males.
2. We predict that smokers with ANRT will have lower MOR BP compared to smokers on PNRT.
Hypotheses: 2a) Women on A-NRT will have higher MOR BP compared with men on A-NRT. 2b) We predict that MOR BP in both A-NRT groups will be elevated relative to nonsmokers. 2c) Women on A-NRT will have higher craving and withdrawal severity than men on A-NRT. 2d) Women on placebo NRT will have higher MOR BP than men on placebo NRT. 2e) Men and women on placebo NRT will have higher MOR BP than men and women on active NRT. 2f) Women on placebo NRT will have higher craving and withdrawal severity than men on placebo NRT.
3. We predict that smokers carrying the minor allele of the A118G polymorphism of the mu opioid receptor gene will have higher MOR BP and greater nicotine craving during PNRT and ANRT.

In our original study, we observed an effect of smoking status on mu-opioid binding potential (MOR BP) in alcohol dependent subjects maintained on nicotine replacement treatment. Specifically, nicotine dependent subjects had significantly lower mu binding potential compared to subjects who were not nicotine dependent. Interestingly, this nicotine effect was in the opposite direction of the effect of alcohol withdrawal in alcohol

Date: October 19, 2017
Protocol Title: Nicotine Dependence, Withdrawal and Replacement Therapy Assessed by PET Imaging
Principal Investigator: Mary E. McCaul, PhD
NCT: 01664741
Application Number: 00-02-03-05 / NA_00036996

dependent subjects. That is, alcohol dependent subjects in alcohol withdrawal had significantly elevated mu opioid binding potential compared with healthy control subjects. Unfortunately, in our original study sample, there were insufficient numbers of healthy control smokers to disentangle the effects of smoking and alcohol withdrawal on MOR BP.

The proposed research will extend this original observation in alcohol dependent subjects through an investigation of mu-opioid system status in healthy nicotine-dependent social drinkers. Smokers will be studied during active smoking status and then during treatment with active versus placebo nicotine replacement therapy. Gender differences in mu-opioid status will be examined. In future research, we will extend this research to alcohol dependent participants.

We also are proposing to examine effects of a relatively common, functional polymorphism of the MOR gene (Exon 1 functional Asn40Asp (A118G) missense single nucleotide polymorphism) on nicotine's modulation of MOR BP. Recent data support the role of A118G polymorphism in smoking initiation and nicotine craving and dependence (Ray, 2006; Lerman, 2004; Munafò 2007, Wang, 2008; Zhang, 2006). Notably, Ray et al (2006) showed a gene by gender interaction on the relative reinforcing value of nicotine (number of nicotine cigarette puffs); among women, only the minor allele was associated with reduced nicotine reward. Two studies have examined the role of the A118G polymorphism in therapeutic responses to NRT, with differing results. One study showed better treatment outcomes in persons expressing the minor allele (Lerman, 2004) whereas the other study showed better treatment outcomes in persons expressing the major allele (Munafò, 2007). Clearly more research is needed on the role of this genotype on smoking maintenance and NRT effectiveness. While the MOR gene is our primary focus, we also will explore opportunities to study genes related to smoking (e.g., nicotinic acetylcholine receptor subunit genes).

4. Study Procedures

a. Study design, including the sequence and timing of study procedures (distinguish research procedures from those that are part of routine care).

Following screening for study eligibility and informed consent, subjects will complete a detailed assessment of alcohol history, nicotine history, psychiatric status, medical history and physical examination (including standard laboratory tests) and assessment of neuroanatomy by MRI prior to the PET procedure. We also will obtain a breath carbon monoxide (CO) reading to confirm smoking/nonsmoking status and a blood spot for determination of phosphatidylethanol (PEth) to confirm social/heavy alcohol use status. When available, we also review the JHH medical record to determine initial and ongoing study eligibility. Eligible subjects will be admitted as inpatients on the Bayview Medical Center CRU.

Healthy Control and Nicotine Dependent Subjects Procedures:

On the morning of the first scan, subjects report to the JHH research office fasting and complete a breathalyzer test for recent alcohol use, a urine test for recent drug use and a pregnancy test. In addition, subjects complete several self-report measures on recent lifestyle and behaviors and current mood. Finally, subjects eat a calorie-controlled breakfast.

Nicotine dependent subjects are allowed to smoke ad lib in a specially designated area until shortly before PET scan 1. Several minutes prior to the scan subjects are administered a controlled dose Quest cigarette (0.6mg nicotine) using the CReSS device to monitor smoking characteristics.

Following the first scan, subjects will be transported to the Bayview Medical Center CRU for their 3-night stay. Subjects will be transported by shuttle or private care service and will be escorted by a research assistant.

Following PET scan 1 and promptly after admission to the CRU subjects will be placed on either active (21 mg) or placebo transdermal nicotine patch. Nicotine craving and withdrawal severity scores will be

Date: October 19, 2017
Protocol Title: Nicotine Dependence, Withdrawal and Replacement Therapy Assessed by PET Imaging
Principal Investigator: Mary E. McCaul, PhD
NCT: 01664741
Application Number: 00-02-03-05 / NA_00036996

obtained twice daily for the duration of the CRU stay. On day 4 following the first PET scan subjects will undergo their second carfentanil scan. They will be transported back to JHH from the Bayview CRU.

This study uses Quest 0.6 mg nicotine cigarettes available commercially. Quest cigarettes utilize a proprietary process that enables the production of nicotine-controlled tobacco that tastes and smokes like the tobacco in other cigarette products. These cigarettes have comparable levels of tar and other cigarette constituents as regular commercially available cigarettes and differ only in nicotine level.

Quest Cigarettes are smoked through sterile mouthpieces of Clinical Research Support System (CRSS) smoking topography machines (Plowshare, Baltimore, MD). The duration of each puff is measured by the CRSS software. Study staff will document the amount of time the participant took to smoke the cigarette and the time smoking was completed. Measures of nicotine reward, craving and withdrawal will be obtained before and after cigarette administration as well as every fifteen minutes during the scan using the Nicotine Effects Scale (Hutchison, 2002).

PET Procedures: All subjects will undergo a MRI in order to obtain structural data, to align the PET imaging planes and to assess for behavioral tolerance of the scanning procedures. PET data will be acquired on HRRT scanner. ^{11}C -carfentanil will be used for mu-opioid receptor scans. An IV catheter is placed. Prior to the scan, a blood specimen for progesterone measurement for menstrual cycle determination is collected using the IV line. Subjects will be positioned in the scanner. Images will be acquired over ninety minutes with an increasing duration. The patient's position will be continuously monitored over the 90-minute scan and any deviation from the original line will be corrected by repositioning.

Time points will be decay corrected by a calculated method and reconstructed using a 26 cm X 26 cm field of view and a 128 X 128 pixel matrix. The scans will be corrected for attenuation immediately prior to acquisition of the emission data using an external source. For calculation of binding potential, images from 35-86 minutes will be summed and smoothed using a 3 X 3 neighborhood averaging filter. Region-of-interest (ROI) placement will be based on the SPGR and T1 MRI images. The MRI images will be co-registered with the PET images in the X-Y plane by translating or rotating the MRI images until the PET and MRI fiducials overlap. ROIs will then be placed on paired left and right structures in the two hemispheres. The ROIs will then be transferred directly to the summed images for sampling. Final values will then be given as the ratio $(\text{ROI} - \text{occipital cortex})/(\text{occipital cortex})$, where occipital cortex represents non-specific binding.

The use of the $(\text{region} - \text{occipital cortex})/(\text{occipital cortex})$ ratio for quantification of ^{11}C -carfentanil studies has been previously validated and used as a reliable index of mu opioid receptor binding by our group for many years (Frost et al, 1989; Frost et al, 1988 Mayberg et al, 1991). This parameter is proportional to the B_{max}/K_D ratios. It permits comparison of data from different patient groups, but does not distinguish B_{max} (receptor number) and K_D (dissociation constant). Data from the right and left sides will be compared to identify right-left asymmetry if it exists. These data will then be used in the statistical analyses described subsequently.

Regions of interest (ROIs) for PET analyses will include: temporal, frontal, parietal, and occipital cortex; cingulate cortex; caudate nucleus; putamen; thalamus; amygdala; and occipital cortex as well as others areas that are identified as involved in nicotine reinforcement and withdrawal. Right and left ROIs will be placed, except in cingulate cortex. Cortical ROIs will be grouped into functional regions such as orbital frontal cortex, prefrontal cortex, anterior temporal neocortex, etc. ANOVA will be used to evaluate the main treatment group effect in the PET data. Relations between individual clinical measures (e.g., peak craving, peak withdrawal score) and the PET results will be assessed using Pearson or Spearman (as appropriate) correlation to assess the relationship between the set of subjective/behavioral measures and the set of regional PET results. We will also examine the use of classificatory methods (discriminant functions, cluster analysis) in analyzing the multivariate data sets, when warranted.

Date: October 19, 2017
Protocol Title: Nicotine Dependence, Withdrawal and Replacement Therapy Assessed by PET Imaging
Principal Investigator: Mary E. McCaul, PhD
NCT: 01664741
Application Number: 00-02-03-05 / NA_00036996

Blood collection for genotype determination: 20cc of blood will be collected at the time of the assessment for DNA extraction and subject genotyping. Blood will be processed into DNA in Dr. Wand's laboratory.

b. Study duration and number of study visits required of research participants.

Healthy control smokers will have three (3) visits: assessment, MRI and CRU admission. Total duration of the CRU stay will be 3 nights.

c. Blinding, including justification for blinding or not blinding the trial, if applicable.

Expectations of drug effectiveness are known to influence clinical outcomes. Subjects will be randomized to active versus placebo patch to control for expectancy effects.

d. Justification of why participants will not receive routine care or will have current therapy stopped.

NRT will be discontinued at the time of discharge from the CRU.

e. Justification for inclusion of a placebo or non-treatment group.

The PNRT group is required to determine the effects of smoking cessation on the primary outcome measure, MOR BP.

f. Definition of treatment failure or participant removal criteria.

Subjects may be terminated from this protocol as a result of several safety concerns. First, if subjects do not comply with CRU rules or study procedures, they will first be warned of possible dismissal and, if noncompliance persists, will be terminated from the protocol. Second, if subjects do not tolerate the NRT, we will first attempt to titrate dose induction; however, if symptoms persist, subjects will be dropped from the study. .

g. Description of what happens to participants receiving therapy when study ends or if a participant's participation in the study ends prematurely.

At the end of the study the nicotine patch is removed and subjects interested in smoking cessation are referred to their doctor.

5. Inclusion/Exclusion Criteria

We typically consent and screen approximately four persons for each subject who meets full study eligibility requirements. This results in a total consented group of 360 persons, including nicotine-dependent and healthy comparison subjects. Subjects will be recruited through the media. We will also recruit subjects who have participated in related studies in our group (NA_00041368; NA_00031348; IRB00033324; IRB00045818) and have consented to future contact for research. We will also ask subjects for permission to retain their information for recruitment via an IRB approved recruitment database for our group (IRB00029464) using the IRB-approved screening database (IRB00029464; Recruitment database for the Integrated Program of Substance Abuse Research [IPSAR]). Minority subjects will be recruited in proportion to their representation in the Baltimore metropolitan area. Men and women will be recruited in equal numbers. To be eligible for study enrollment, nicotine-dependent subjects (N=60 who complete the protocols) must be 21 - 60 years old, meet DSM-5 criteria for tobacco use disorder, and be actively smoking 10 – 40 cigarettes daily. Subjects are ineligible who: 1) meet DSM-5 criteria for

Date: October 19, 2017
Protocol Title: Nicotine Dependence, Withdrawal and Replacement Therapy Assessed by PET Imaging
Principal Investigator: Mary E. McCaul, PhD
NCT: 01664741
Application Number: 00-02-03-05 / NA_00036996

current alcohol or drug use disorder (excluding nicotine, marijuana and caffeine); 2) meet DSM-5 criteria for life psychotic disorder, current mood or anxiety disorders and are in need of or currently undergoing pharmacotherapy. Participants who are current taking a SSRI or SNRI medication but do not meet current diagnostic criteria for a mood or anxiety disorder will be included; 3) are pregnant or lactating; 4) are currently experiencing a serious medical condition that would place them at risk or interfere with study participation; 5) have a history of severe allergies, multiple adverse drug reactions or known allergy to NRT; 8) have a Shipley vocabulary score below the 5th grade reading level; 9) abnormal MRI scan; 10) are HIV positive (to rule out potential neurological sequelae); 11) significant CNS disease; 12) significant history of seizure disorder or closed head trauma; 13) neuroendocrine disorder; or 14) treatment with opioid agonists or antagonists within the last six months.

In addition to recruitment of nicotine-dependent subjects, age- and race-matched nonsmokers (N=30 who complete the protocols) will be recruited as a comparison sample. This comparison sample is needed to determine magnitude and direction of opioid system derangements in nicotine-dependent subjects relative to age-matched controls. These subjects will be recruited through the media and will include equal numbers of men and women.

Table 1: Inclusion Criteria	Nicotine-Dependent Subjects	Healthy Nonsmokers
Males and females	X	X
Age 21 - 60 (inclusive)	X	X
Meet DSM-5 criteria for tobacco use disorder	X	
Actively smoking 10 – 40 cigs/day	X	

Table 2: Exclusion Criteria	Nicotine-Dependent Subjects	Healthy Control Nonsmokers
Abnormal MRI scan	X	X
Weight > 350 lbs	X	X
Currently experiencing serious medical condition that would place subject at risk or interfere with study participation	X	X
Meet DSM-5 criteria for life psychotic disorder, current mood or anxiety disorders and are in need of or currently undergoing pharmacotherapy. Participants who are current taking a SSRI or SNRI medication but do not meet current diagnostic criteria for a mood or anxiety disorder will be included;	X	X
DSM-5 criteria for current alcohol use disorder	X	X
DSM-5 criteria for current substance use disorder (non-alcohol) (excluding caffeine, marijuana and nicotine); with the exception of marijuana, subjects who report drug use in last 30 days or have a positive urine toxicology screen at initial assessment will be given additional opportunities to qualify for the study.	X	X
For women, amenorrhea; pregnancy, planned pregnancy or lactation; postmenopausal women are eligible	X	X
Hx of any central nervous system disorder; or presence of a seizure disorder or closed head trauma	X	X
Hx of severe allergies, multiple adverse drug reactions or known allergy to NRT	X	X
HIV positive	X	X
Neuroendocrine disorder	X	X
Shipley vocabulary score < 18, corresponding to a 5 th grade reading level	X	X
Treatment with antidepressants, neuroleptics, sedative hypnotics, glucocorticoids, appetite suppressants, opiates, or dopamine	X	X

Date: October 19, 2017
Protocol Title: Nicotine Dependence, Withdrawal and Replacement Therapy Assessed by PET Imaging
Principal Investigator: Mary E. McCaul, PhD
NCT: 01664741
Application Number: 00-02-03-05 / NA_00036996

medications within the last 6 months		
Smoking cessation treatment within last 6 months	X	X

We exclude potential subjects with a Shipley score below the 5th grade reading level because of concerns about their ability to adequately participate in the study procedures. Many of our behavioral/subjective measures are self-administered and require basic literacy skills of the subjects. If subjects are not at a 5th grade level, they have difficulty responding accurately to the study questionnaires.

Historically, we have not had language/hearing-impaired individuals apply for study participation. If a language or hearing impaired individual did apply, s/he would be ruled out on this basis.

Consent Issues:

Consent is obtained at the initial face-to-face interview in the research office by the IRB-approved research staff. Subjects are familiarized with the scope of study procedures and time commitment during the initial telephone contact with research staff. They read and are asked to sign the consent document at the start of the face-to-face assessment session. If subjects have questions or concerns, they are free to reschedule the assessment and delay signing the consent document.

We take several precautions to ensure that subjects are fully informed and understand the risks of our research protocols. When individuals first call in response to our advertisements, we provide a brief overview of the study to acquaint them with the nature and primary requirements of the study. If the individual remains interested and reports for the in-person interview, we obtain a breath alcohol level to determine that the potential subject is alcohol-free at the time we obtain informed consent.

6. Drugs/ Substances/ Devices

a. The rationale for choosing the drug and dose or for choosing the device to be used.

Active nicotine patch (21 mg) is the standard of care for management of nicotine withdrawal.

b. Justification and safety information if FDA approved drugs will be administered for non-FDA approved indications or if doses or routes of administration or participant populations are changed.

Not Applicable.

c. Justification and safety information if non-FDA approved drugs without an IND will be administered.

Quest 0.6 mg nicotine and 0.05 mg nicotine ("denicotinized") cigarettes are available commercially. These are not regulated by the FDA.

Quest cigarettes utilize a proprietary process that enables the production of nicotine-free tobacco that tastes and smokes like the tobacco in other cigarette products. These cigarettes have comparable levels of tar and other cigarette constituents as regular commercially available cigarettes and differ only in nicotine level.

7. Study Statistics

a. Primary outcome variable.

Part I. CRU Stay:

Date: October 19, 2017
Protocol Title: Nicotine Dependence, Withdrawal and Replacement Therapy Assessed by PET Imaging
Principal Investigator: Mary E. McCaul, PhD
NCT: 01664741
Application Number: 00-02-03-05 / NA_00036996

PET-derived regional mu opioid receptor binding by ROI and SPM

Presence or absence of NRT

Subjective/behavioral measures on the CRU:

- peak analog craving
- peak nicotine withdrawal score

b. Secondary outcome variables.

Not Applicable.

c. Statistical plan including sample size justification and interim data analysis.

The proposed sample sizes of 60 nicotine-dependent subjects and 30 non-smoking control subjects provide power in excess of .80 to detect a medium effect size in ANOVA analyses of group differences and a correlation of .25 at the 95% confidence level.

Analysis of Variance (or, if necessary, Covariance to adjust for baseline group differences) will be used to examine differences between nicotine dependent and nonsmoking subjects on PET, neuroendocrine and subjective measures.

Multiple regression analysis will be the primary data analytic method to examine the relationships between the predictor measures (gender, NRT (active vs placebo), nicotine withdrawal severity and craving) and opioid receptor binding potential.

Assessment Measures for Statistical Analysis:

This study will not involve the use of a data monitoring board since the therapy to be studied is the current FDA approved standard of care. Should a subject experience a serious adverse event, this will be immediately reported to the JCCI and to our NIDA project officer. Also, should we observe a trend in the occurrence of adverse events related to one or more study procedures, we will examine the need to modify the study protocol to further enhance subject safety. Finally, in the annual renewal report for this project, adverse events will be summarized.

There are no plans for interim data analyses.

d. Early stopping rules.

Pharmacotherapy will be discontinued based on adverse drug reactions.

8. Risks

a. Medical risks, listing all procedures, their major and minor risks and expected frequency.

This study involves several separate procedures, each of which entails some risk of discomfort or medication side-effects. First, PET risks include a risk of bleeding or infection from the venous catheter, which is placed. The effective radiation dose to the whole body for two studies is below the yearly occupational limit for a radiation worker. Fourth, the nicotine patch dose selected for study in this protocol is FDA-approved and has been shown to have a low incidence rate of serious side-effects or adverse events in clinical trials with nicotine-dependent patients. Less than 5% of smokers have to stop

Date: October 19, 2017
Protocol Title: Nicotine Dependence, Withdrawal and Replacement Therapy Assessed by PET Imaging
Principal Investigator: Mary E. McCaul, PhD
NCT: 01664741
Application Number: 00-02-03-05 / NA_00036996

using a nicotine replacement product because of side effects. Side effects of nicotine patches may include: skin rash at the location of the patch; sleep problems when using a 24-hour patch, such as having trouble sleeping or having especially vivid dreams. On rare occasions, there have been reports of severe allergic reactions (rash; hives; itching; difficulty breathing; tightness in the chest; swelling of the mouth, face, lips, or tongue); fast or irregular heartbeat; mouth, teeth, or jaw problems; pounding in the chest; severe diarrhea, dizziness, nausea, vomiting, or weakness. The relatively brief period of NRT administration in this study (4 days) should minimize the likelihood of any serious problems developing.

Several additional risks occur throughout the course of the study. There is a slight risk of bruising and irritation at the site of blood draws. This risk is minimized through the use of sterile equipment and well-trained medical personnel. There is a risk of distress or personal discomfort elicited during assessment or psychosocial treatment sessions. This risk is minimized by the use of standardized assessment and treatment procedures that are widely and successfully used in clinical settings. Also, all study staff are trained in nonjudgmental interview techniques and crisis intervention procedures. If serious psychological concerns (e.g., suicide risk) arise, staff are trained to refer these to Dr. McCaul, a licensed psychologist.

If subjects are required to have an x-ray examination of the head/eyes because of a history of working with metal or a history of metal in the subject's head/eyes, the subject will be exposed to additional radiation. The total additional amount of radiation subjects will receive from the x-ray examination is 0.01 rem. Naturally occurring radiation (cosmic radiation, radon, etc) produces whole body radiation exposures of about 0.3 rem per year. Occupationally exposed individuals are permitted to receive whole body exposure of 5 rem per year.

¹¹C-Carfentanil: ¹¹C-Carfentanil is a carbon 11 labeled opioid agonist radiopharmaceutical used for imaging the mu subtype opioid receptor. ¹¹C-Carfentanil is currently the only validated PET ligand for studying *in vivo* the mu-opioid receptors in humans (Henriksen et al, 2005) and has been used safely in numerous recent studies (Bencherif et al, 2002; Bencherif et al, 2004b; Liberzon et al, 2002; Liberzon et al, 2007; Weerts et al, 2008; Weerts et al, 2011; Zubieta et al, 2001; Zubieta et al, 2002; Zubieta et al, 2003; Zubieta et al, 2005). There is a single report of an adverse event; one subject experienced an idiosyncratic allergic reaction which may have been to carfentanil or Hydromorphone, but the source could not be confirmed (Greenwald et al. 2003).

Radioactive Drug and Radiation Exposure: The radioligand ¹¹C-carfentanil was developed at Johns Hopkins by Dr. James Frost in the 1980s. ¹¹C-carfentanil has been administered to numerous human subjects over the years without any known incident of a subject experiencing an adverse effect. Based on other ¹¹C-carfentanil studies (Weerts et al, 2008; Weerts et al, 2011; Zubieta et al, 2000; Zubieta et al, 2003; Greenwald et al, 2003) and personal communication with Drs. Frost and Zubieta, the dose of carfentanil that will be administered in this study is 0.05 µg/kg. At this dose, we have not observed any adverse effects. At larger doses of ¹¹C-carfentanil, opiate-like effects such as drowsiness, a good or bad change in mood state, nausea, vomiting, itching or a mild headache could occur. However, at the mass dose being administered, subjects may possibly experience lightheadedness (J. Frost, personal communication).

Participants are exposed to 0.644 rem in this study, less than 5 rem effective dose per year currently approved occupational limit. The Clinical Radiation Research Committee of Johns Hopkins University has increasingly employed the ICRP method of effective dose calculation (Annals ICRP 1987). This method consists of weighing various organs by dosimetry relative to their radiosensitivity to provide a single effective dose for the total body. Johns Hopkins University has taken the position that effective total body dose should typically remain below 5 rem to remain within the occupational limit per year. For ¹¹C-carfentanil, the effective dose equivalent is 16 mrem/mCi; so for two 20 mCi intravenous injections, the total radiation exposure is 639 mrem or 0.639 rem. All subjects will undergo a brain transmission scan after each PET scan and will receive an additional 2.6 mrem of radiation exposure from each transmission scan. Thus the total radiation dose (effective dose) from PET scanning procedures will be

Date: October 19, 2017
Protocol Title: Nicotine Dependence, Withdrawal and Replacement Therapy Assessed by PET Imaging
Principal Investigator: Mary E. McCaul, PhD
NCT: 01664741
Application Number: 00-02-03-05 / NA_00036996

644 mrem or 0.644 rem. If participants are required to have the skull x-rays performed, the radiation exposure will increase by 0.01 rem. Thus the total radiation exposure for both procedures will be 0.654 rem. The specific radiation absorbed doses to individual organs, provided by Dr. James Frost from his IND (#IND 24,068) for ^{11}C -carfentanil, are listed in the accompanying radiation form.

The maximum radiation exposure subjects will receive from this research study is approximately 0.654 rem. This is more than the 0.3 rem that the average person in the United States gets each year from natural sources like the sun, outer space, air, food and soil. It is less than the 5 rem of radiation that is allowed each year for people who are exposed to radiation in their jobs. This research study includes exposure to radiation from x-rays or gamma rays. This radiation exposure is for research purposes only and is not part of the subject's medical care. X-rays and gamma rays from natural or medical sources can damage the genetic material (DNA) in the subject's cells. At low doses, the body is usually able to repair the damage.

Dean Wong, MD PhD is a Professor of Radiology, Psychiatry and Environmental Health Sciences at Johns Hopkins University and a Board-Certified Nuclear Medicine Physician. He will have overall responsibility of the PET scanning component of the project. He will supervise all Hopkins staff and the PET technologist who will acquire the PET scans. Dr. Wong will supervise all PET scan acquisitions. Dr. Wong's faculty and staff will analyze the PET data.

Blood draws and urine collection: We will collect blood for nicotine-related genetic analyses. Blood chemistries and urinalysis are performed at assessment. We also collect a blood specimen for progesterone measurement for menstrual cycle determination. Urinalysis for drug toxicology assessment is performed to identify illicit drug use. These procedures involve minimal risks, such a slight risk of discomfort at the intravenous site. A small amount of bleeding under the skin will produce a bruise in about 5% of cases. The risk of temporary clotting of the vein is about 1%. The risk of infection or significant blood loss is less than 1 in 1000. In rare cases, fainting could occur.

Genetic information: The genetic SNP variants to be studied are not currently known to cause any medical conditions and are being examined for the purposes of this experiment only. Thus, the risks, such as loss of privacy, resulting from our ascertainment of genetic information are extremely low. DNA will be banked for future analyses based on new research findings; samples are stored using subject ID only.

Risk/Benefit Ratio: The primary risk to participants is the radiation exposure. The effective dose of two 20 mCi injections of ^{11}C -carfentanil and two transmission scans is 0.644 rem. This radiation exposure is 4.356 rem or 87% less than the annual radiation exposure limit of 5 rem for occupationally exposed persons. Given the percentage of exposure is significantly lower than the annual limit and participants will be excluded from the study if they have had previous radiation exposures that in addition this study would place above the annual limit, the risks are perceived to within acceptable limits.

b. Steps taken to minimize the risks.

Some subjects may develop a skin rash at the location of the nicotine patch. This may be a reaction either to the sticky backing on the patch or to the nicotine. People who experience significant allergic reaction to the patch will be discontinued and will be provided with medical care to reverse symptoms. Moving the patch to a different part of your body or using a nonprescription antihistamine cream, ointment, or gel (such as Benadryl) may relieve some of the discomfort.

c. Plan for reporting unanticipated problems or study deviations.

All unanticipated problems or study deviations will be reported promptly to the IRB.

d. Legal risks such as the risks that would be associated with breach of confidentiality.

Date: October 19, 2017
Protocol Title: Nicotine Dependence, Withdrawal and Replacement Therapy Assessed by PET Imaging
Principal Investigator: Mary E. McCaul, PhD
NCT: 01664741
Application Number: 00-02-03-05 / NA_00036996

Subjects will be assigned a code number and all blood samples will be analyzed using code numbers and not names. However, a master list of names and code numbers will be retained by the Investigators and will be stored separately from the study data.

In any study that involves questions about hazardous or possibly illegal behaviors, there is a risk of breach of confidentiality by study staff. This risk is minimized by all staff being carefully trained in the protection of confidentiality and their participation in regular updates of patient/subject confidentiality. Further protections include the use of subject numbers to code all data forms for computer entry and storage, and reporting of study findings using group data only. All data are stored with code numbers only; the master list of names and associated code numbers is maintained by the Investigators separately from the data files.

e. Financial risks to the participants.

All costs will be covered by NIH grants.

9. Benefits

a. Description of the probable benefits for the participant and for society.

This study is conducted for the advancement of science. It is clinically and scientifically important to understand perturbations of the opioid system associated with tobacco use disorder and to identify predictors of clinical effectiveness of nicotine replacement therapy.

10. Payment and Remuneration

a. Detail compensation for participants including possible total compensation, proposed bonus, and any proposed reductions or penalties for not completing the protocol.

Possible Total Compensation:

All enrolled subjects will receive \$25/day as their earnings for each CRU day. While the duration of the CRU stay may vary slightly across subjects (e.g., based on availability of the PET imaging Center), we expect subjects to average 4 days (3 nights) on the unit. Subjects who complete all study procedures will receive a \$100 bonus. Total compensation for study participation includes:

Payments for study completers:

Assessment	\$ 75.00	
MRI	\$ 50.00	
PET scan(s) - \$125/scan X 2 scans	\$250.00	
3 CRU nights X \$25/night	\$ 75.00	
2 additional CRU nights X \$25/night	\$ 50.00	(if needed)
Completion Bonus	\$100.00	
Maximum total subject payment	\$600.00	

Reductions or Penalties for Not Completing:

Subjects who are terminated for noncompliance or who drop out of the study will be paid only the amount earned to date for completed procedures, CRU stay or outpatient visits. They will forfeit future possible earnings and bonus payments. To eliminate any financial incentive for early termination, subjects who are noncompliant or drop out will not receive payment until the day when they were scheduled to

Date: October 19, 2017
Protocol Title: Nicotine Dependence, Withdrawal and Replacement Therapy Assessed by PET Imaging
Principal Investigator: Mary E. McCaul, PhD
NCT 01664741
Application Number: 00-02-03-05 / NA_00036996

complete their study procedures. If subjects are terminated by the Investigators for safety reasons, they will be paid the amount earned to date for completed procedures, CRU stay or outpatient visits. Additionally, they will still be eligible for bonus payments prorated for length of study participation.

11. Costs

- a. Detail costs of study procedure(s) or drug (s) or substance(s) to participants and identify who will pay for them.**

Not Applicable.

Date: October 19, 2017
Protocol Title: Nicotine Dependence, Withdrawal and Replacement Therapy Assessed by PET Imaging
Principal Investigator: Mary E. McCaul, PhD
NCT: 01664741
Application Number: 00-02-03-05 / NA_00036996

12. References

1. Bencherif B, Fuchs PN, Sheth R, et al: Pain activation of human supraspinal opioid pathways as demonstrated by [11C]-carfentanil and positron emission tomography (PET). *Pain* 2002; 99 (3): 589-598.
2. Bencherif B, Wand GS, McCaul ME, et al: Mu-opioid receptor binding measured by [11C]carfentanil positron emission tomography is related to craving and mood in alcohol dependence. *Biol Psychiatry* 2004b; 55 (3): 255-62.
3. Cohen MR, Cohen RM, Pickar D, et al: High-dose naloxone infusions in normals. Dose-dependent behavioral, hormonal, and physiological responses. *Arch Gen Psychiatry* 1983; 40 (6): 613-619.
4. Frost JJ, Douglass KH, Mayberg HS, et al: Multicompartmental analysis of [11C]-carfentanil binding to opiate receptors in humans measured by positron emission tomography. *J Cereb Blood Flow Metab* 1989; 9 (3): 398-409.
5. Frost JJ, Mayberg HS, Fisher RS, et al: Mu-opiate receptors measured by positron emission tomography are increased in temporal lobe epilepsy. *Ann Neurol* 1988; 23 (3): 231-237.
6. Frost JJ: Personal communication on the adverse effects of ¹¹C-carfentanil. 2006.
7. Greenwald MK, Johanson CE, Moody DE, et al: Effects of Buprenorphine Maintenance Dose on mu-Opioid Receptor Availability, Plasma Concentrations, and Antagonist Blockade in Heroin-Dependent Volunteers. *Neuropsychopharmacol* 2003; 28: 2000-2009.
8. Henriksen G, Platzer S, Marton J, et al: Syntheses, biological evaluation, and molecular modeling of 18F-labeled 4-anilidopiperidines as mu-opioid receptor imaging agents. *J Med Chem* 2005; 48 (24): 7720-7732.
9. Hutchison KE, LaChance Niaura HR, et al: The DRD4 VNTR influences reactivity to smoking cues. *J Abnorm Psychol* 2002; 111 (1): 134-143.
10. Lerman C, Wileyto EP, Patterson F, Rukstalis M, Audrain-McGovern J, Restine S, Shields PG, Kaufmann V, Redden D, Benowitz N, Berrettini WH: The functional mu opioid receptor (OPRM1) Asn40Asp variant predicts short-term response to nicotine replacement therapy in a clinical trial. *Pharmacogenomics J* 2004; 4 (3):184-192.
11. Liberzon I, Taylor SF, Phan KL, et al: Altered Central mu-Opioid Receptor Binding After Psychological Trauma. *Biol Psychiatry* 2007; 61 (9): 1030-1038.
12. Liberzon I, Zubieta JK, Fig LM, et al: mu-Opioid receptors and limbic responses to aversive emotional stimuli. *Proc Natl Acad Sci USA* 2002; 99 (10): 7084-7089.
13. Mayberg HS, Sadzot B, Meltzer CC, et al: Quantification of mu and non-mu opiate receptors in temporal lobe epilepsy using positron emission tomography. *Ann Neurol* 1991; 30 (1): 3-11.
14. Munafò MR, Elliot KM, Murphy MF, Walton RT, Johnstone EC: Association of the mu-opioid receptor gene with smoking cessation. *Pharmacogenomics J* 2007; 7 (5): 353-361.
15. Ray R, Jepson C, Patterson F, Strasser A, Rukstalis M, Perkins K, Lynch KG, O'Malley S, Berrettini WH, Lerman C: Association of OPRM1 A118G variant with the relative reinforcing value of nicotine. *Psychopharmacology (Berl)* 2006; 188 (3): 355-363.
16. Task Group of Committee 2: Radiation Dose to Patients from Radiopharmaceuticals. *Annals ICRP* 1987; 18 (1-4): 1-29.

Date: October 19, 2017
Protocol Title: Nicotine Dependence, Withdrawal and Replacement Therapy Assessed by PET Imaging
Principal Investigator: Mary E. McCaul, PhD
NCT: 01664741
Application Number: 00-02-03-05 / NA_00036996

17. Wang Z, Ray R, Faith M, Tang K, Wileyto EP, Detre JA, Lerman C: Nicotine abstinence-induced cerebral blood flow changes by genotype. *Neurosci Lett* 2008; 438 (3): 275-280.
18. Weerts EM, Kim YK, Wand GS, et al: Differences in delta- and mu-Opioid Receptor Blockade Measured by Positron Emission Tomography in Naltrexone-Treated Recently Abstinent Alcohol-Dependent Subjects. *Neuropsychopharmacol* 2008 33: 653-665.
19. Weerts EM, Wand GS, Kuwabara H, et al: PET imaging of mu- and delta-opioid receptor binding in alcohol dependent and healthy subjects. *Alcohol Exp Clin Res* 2011. In Press.
20. Zhang L, Kendler KS, Chen X: The mu-opioid receptor gene and smoking initiation and nicotine dependence. *Behav Brain Funct* 2006; Aug 4; 2: 28.
21. Zubieta J, Greenwald MK, Lombardi U, et al: Buprenorphine-induced changes in mu-opioid receptor availability in male heroin-dependent volunteers: a preliminary study. *Neuropsychopharmacol* 2000; 23 (3): 326-334.
22. Zubieta JK, Bueller JA, Jackson LR, et al: Placebo Effects Mediated by Endogenous Opioid Activity on μ -Opioid Receptors. *J Neurosci* 2005; 25 (34): 7754 –7762.
23. Zubieta JK, Ketter TA, Bueller JA, et al: Regulation of human affective responses by anterior cingulate and limbic mu-opioid neurotransmission. *Arch Gen Psychiatry* 2003; 60 (11): 1145-1153.
24. Zubieta JK, Smith YR, Bueller JA, et al: Regional mu opioid receptor regulation of sensory and affective dimensions of pain. *Sci* 2001; 293 (5528): 311-315.
25. Zubieta JK, Smith YR, Bueller JA, et al: Mu-Opioid Receptor-Mediated Antinociceptive Responses Differ in Men and Women. *J Neurosci* 2002; 22 (12): 5100–5107.

Date: October 19, 2017
Protocol Title: Nicotine Dependence, Withdrawal and Replacement Therapy Assessed by PET Imaging
Principal Investigator: Mary E. McCaul, PhD
NCT: 01664741
Application Number: 00-02-03-05 / NA_00036996

APPENDIX 1: List of Current Study Instruments

- 90-day Time Line Follow Back Assessment
- Alcohol Use Disorders Identification Test (AUDIT)
- Analog craving scales
- Beck Depression Scale
- Breath Alcohol Concentration (BAC)
- Fagerstrom Test for Nicotine Dependence (FTND)
- Minnesota Nicotine Withdrawal Scale (MNWS)
- Nicotine Effects Scale
- Questionnaire of Smoking Urges (QSU-Brief)
- Alcohol and Nicotine sections of the Semi-structured Assessment for the Genetics of Alcoholism
- Shipley Vocabulary Questionnaire
- Michigan Nicotine Reinforcement Questionnaire
- Health Checklist
- Registration Sheet
- Menstrual Cycle Questionnaire
- M.I.N.I. International Neuropsychiatric Interview 7.0 (01/01/14 version) – IPSAR version 2014.04.16
- Life Events Checklist for DSM 5 (LEC-5)

Race and ethnicity will be determined by self-report using NIH categories.